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14. ABSTRACT

Increasing evidence suggests that breast cancer risk is determined early in life. Mammographic density has been used as a biomarker for breast cancer risk because of its strong association with breast cancer. However, this screening method cannot be used in young women and girls because the risk of X-ray based mammograms outweighs potential benefits in that age group. In contrast, Dual Energy X-ray Absorptiometry (DXA) has extremely low radiation. The specific aims of this project among adult women and their adolescent daughters were to [1] Correlate breast density measured by DXA with mammographic density among adult women; [2] Compare the association of known breast cancer risk factors with breast density from DXA scans to their association with mammographic density; [3] Assess DXA breast density by Tanner stage of breast maturation among adolescent girls [4] Relate DXA breast density to other observable measures of pubertal maturation; and [5] Examine the relation between breast density measured by DXA in mothers and daughters.

We recruited 101 mothers 30 years and older and their daughters aged 10-16 years, representing the multiethnic population of Hawaii. Comparison of mothers' DXA breast images with mammography showed a strong correlation of percent density between the two methods (r=0.76, p <0.0001). Associations with common breast cancer risk factors show similar patterns for DXA and mammographic densities; in particular, the inverse associations with BMI and age at menarche were evident for both methods. Evaluation of daughters' DXA breast measures in association with pubertal development characteristics and adiposity suggest a linear relation (p<0.001) of breast volume and fibroglandular volume (FGV) with Tanner breast stages. In addition, breast volume and FGV were positively related to measures of body fat. On the other hand, %FGV show an inverse association with all measures of body fat. Comparison of daughters' DXA measures with mothers' DXA measures indicated that total breast area, total breast volume, and FGV are higher in mothers than in daughters, while mean % FGV was higher in daughters than in mothers. We observed statistically significant correlations between breast measures of mothers and daughters except for %FGV; the association was stronger for girls who had reached Tanner breast stages 4 & 5. These results indicate that the heritability of breast volume and amount of dense tissue is already visible in adolescence, but an association for percent density may only become apparent at a later time. The current findings suggest the potential use of DXA as a low-radiation option in evaluating longitudinal changes in breast tissue composition in combination with body fat measures and other heritable risk factors.

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   Manuscript 1: *Comparison of breast density measured by dual energy x-ray Absorptiometry (DXA) with mammographic density among adult women in Hawaii (Cancer Epidemiology, in press).*
   Manuscript 2: *Body fat and menarche are associated with breast density in multiethnic adolescent girls (American Journal of Human Biology, under review).*
   Manuscript 3: *Comparison of breast measures between mothers and adolescent daughters (Breast Cancer Research, under review).*

(4) Introduction

Increasing evidence suggests that breast cancer risk is determined early in life. However, use of mammography as a screening method is contraindicated in young women and girls because the risk of X-ray exposure outweighs potential benefits in that age group. In contrast, Dual Energy X-ray Absorptiometry (DXA) has extremely low radiation and is commonly available in medical care settings. The rationale of this project is that predictors of breast cancer risk may be observed during pubertal development in girls. Our hypotheses are as follows:

1. DXA imaging can provide a valid assessment of breast density in adult women and in young girls.
2. DXA assessed breast density is associated with indicators of pubertal maturation and with known breast cancer risk factors.
3. Due to its strong genetic component, breast density obtained from the DXA scans is correlated between mothers and adolescent daughters.

The specific aims of this project among adult women and adolescent girls, who are recruited as mothers and daughters, are to:

1. Correlate breast density measured by DXA with mammographic density among adult women.
2. Compare the association of known breast cancer risk factors with breast density from DXA scans to their association with mammographic density.
3. Assess DXA breast density by Tanner stage of breast maturation among adolescent girls.
4. Relate DXA breast density to other observable measures of pubertal maturation (e.g., height and menarche).
5. Examine the relation between breast density measured by DXA in mothers and daughters.

We aimed to recruit a total of 100 adult women with daughters between 10-16 years of age. Based on the ethnic distribution of Hawaii's population, we expected that approximately half of the study subjects will be of Asian (primarily Japanese, Chinese, and Filipino) and Pacific Islander descent.

(5) Body

During the 3 years of the funding period, we have accomplished all the tasks as outlined in the approved Statement of Work.

Task 1. Study plan and procedures. We completed participant recruitment and all activities related to data collection during the previous year. There were no new activities to report for the current year. We have completed the data analysis and continue to work with our collaborators at the University of California at San Francisco (UCSF) and at Kaiser Permanente Hawaii to finalize the manuscripts.

Task 2. Subject recruitment. All subject recruitment was completed during year 2. No additional recruitment activities were conducted in the current year.

Task 3. Conduct study visits. All study visits were completed during year 2. No additional visits were conducted this year.

Task 4. Perform DXA scans. We completed DXA scans of all participants during year 2. No DXA scans were performed this year.

Task 5. Perform Breast Density Analysis. We obtained screening mammogram films for all except one mother, which was recorded as missing data. Mammogram films were scanned on a Kodak LS80 digitizer. Percentage density was calculated as the ratio of the dense area to the total area after delineating total and dense breast areas of the breast. In addition, analysis of all DXA breast images was completed, and breast area, breast volume, absolute fibroglandular tissues (FGV) and % FGV were calculated.

Task 6. Data Management and Analysis. Data entry was completed including demographic data, health and menstrual/reproductive information, anthropometric measures, and Tanner stages for girls. The database was merged with mammographic density data and DXA scan data for statistical analysis using SAS Version 9.2. We conducted a comparison of mothers' DXA breast images and mammography; the manuscript was accepted for publication in *Cancer Epidemiology* (Appendix 1). We also completed the evaluation of daughters' DXA breast measures in association with pubertal development characteristics and adiposity. A manuscript summarizing the results is currently under review with *the American Journal of Human Biology* (Appendix 2). Moreover, we finalized the comparison of daughters' breast measures with mothers' and submitted a manuscript to *Breast Cancer Research* (Appendix 3).

(6) Key Research Accomplishments

- Based on the comparison of breast density measured by DXA with mammographic density, we demonstrated that DXA imaging can provide a valid assessment of breast density in adult women.
- DXA breast measures in girls were associated with indicators of pubertal maturation and body fat composition also measured by DXA.
- We observed that breast density obtained from the DXA scans is correlated between mothers and adolescent daughters, in particular breast volume and absolute amount of fibroglandular tissue but not in percent density.
- Our findings support potential use of DXA as a low-radiation option in evaluating longitudinal changes in breast tissue composition in combination with body fat measures and other heritable risk factors.

(7) Reportable Outcomes

During the 3-year grant period, we completed all of the specific aims originally proposed in the grant application. We conducted analyses comparing DXA breast images with mammographic images in adult women. Results showed a strong correlation of percent density between the two methods (r=0.76, p <0.0001). Associations with common breast cancer risk factors showed similar patterns for DXA and mammographic densities; in particular, the inverse associations with BMI and age at menarche were evident for both methods. A manuscript titled "Comparison of breast density measured by dual energy x-ray Absorptiometry (DXA) with mammographic density among adult women in Hawaii" has been accepted for publication in *Cancer Epidemiology* and is included in the Appendix.

We also completed the evaluation of daughters' DXA breast measures in association with pubertal development characteristics and adiposity; a manuscript summarizing the results is currently under review with *the American Journal of Human Biology* and included in the Appendix. We found a linear relation (p<0.001) of breast volume and absolute FGV with Tanner breast stages. In addition, breast volume and absolute FGV were positively related to measures of body fat when girls were stratified into quartiles of body fat measured as BMI, BMI-for-age percentile and % total body fat. On the other hand, %FGV showed an inverse association with all measures of body fat. These results suggest that the heritability of breast volume and amount of dense tissue is already visible in adolescence, but an association for percent density may only become apparent at a later time.

Finally, we recently finalized the comparison of DXA measures between mothers and daughters. Total breast area, total volume and absolute FGV were higher in mothers than in daughters, while mean %FGV was higher in daughters than in mothers. We observed statistically significant correlations between breast measures of mothers and daughters except for % FGV; the association was stronger for girls who had reached Tanner breast stages 4 & 5). In a multiple regression analysis of all mother-daughter pairs, breast volume (p = 0.03) and absolute FGV (p <0.01) of the mothers remained modestly associated with the respective measures of their daughters, but no association was observed for % FGV (p = 0.82). When limited to the more mature girls with Tanner breast stages 4 & 5, the regression estimates for breast volume and absolute FGV of the mothers improved to 0.18 and 0.63 while the estimate for % FGV was -0.24 (p = 0.20). We recently submitted a manuscript to *Breast Cancer Research*, which is also included in the Appendix. We also presented some of the key findings from the current project at scientific conferences as follows:

1. **Maskarinec G, Morimoto Y, Daida Y, Laidevant A, Malkov S, Shepherd JA, Novotny R.** Comparison of breast density measured by dual energy x-ray Absorptiometry (DXA) with mammographic density among multiethnic adult women in Hawaii. 101st Annual Meeting of the American Association for Cancer Research, Washington, DC, 4/2010.
2. **Novotny R, Daida Y, Morimoto Y, Shepherd J, Maskarinec G.** Breast Density in Multiethnic Adolescent Girls. 16th Annual HMO Research Network Conference, Austin, TX, 3/2010.

(8) Conclusions

The current study's findings provide supporting data on DXA breast scans as a method to evaluate breast cancer risk in women and young girls. The procedure was well accepted and tolerated by adult women and adolescent girls. The results observed in this cross-sectional study suggest potential uses of DXA as a low-radiation option in evaluating longitudinal changes in breast tissue composition in combination with body fat measures and other breast cancer risk factors. DXA is widely available in medical care settings and should be further explored as a technique that may provide a unique tool to screen for breast cancer risk in early life and aid in developing prevention strategies.

(9) References No articles cited.

(10) Supporting Data No supporting data attached.

(11) Personnel

1. **Gertraud Maskarinec, MD, PhD** as Principal Investigator;
2. **Rachel Novotny, PhD, RD** as Principal Investigator;
3. **John Shepherd, PhD** as Co-investigator (responsible for all aspects of the data DXA breast densitometry acquisition, algorithm development, phantom development, interpretation of results and supervision of other UCSF personnel);
4. **Serghei Malkov, PhD** (responsible for workstation development and continued DXA algorithm and phantom development);
5. **Aurelie Laidevant** as Research Assistant (responsible for analyzing the DXA data for all subjects and phantom scans);
6. **Yihe Daida, MS** as Project Manager (responsible for coordinating study recruitment and mammography film acquisition, Kaiser IRB and overseeing data tracking and management);
7. **Yukiko Morimoto, MS, RD** as Project Coordinator (responsible for collecting and processing mammogram films and overall data management and statistical analysis);
8. **Mary Sherman, RT,** (responsible for building and maintaining a SAS database of all DXA measures and for the interpretation of the QC data;
9. **Jane Yakuma, RT** for obtaining informed consent/assent, demographic questionnaire, DXA scan and Tanner staging;
10. **Kathryn Mau** as Research Assistant (responsible for mailing recruitment letters, calling, screening and scheduling participants);
11. **Darlene Hobbs** substituted for Kathy Mau when Kathryn was not available.
12. **Jeff Wang,** as Project Manager at UCSF (responsible for all study operations, resolves problems and coordinates all aspects of the project);
13. **Lorena Marquez** as Research Assistant at UCSF? (responsible for receipt of all data, preparation of the site manuals, and communication with the DXA site for data transfers, review all scans for validity, involve in further phantom and algorithm development, data analysis, and presentation and preparation of results);
14. **Aleli Vinoya** as Data Manager at Kaiser (responsible for pulling potential recruits from the database and tracking database);
15. **Rosina Everette** replaced Aleli for a period of time when Aleli was not available.

(12) Appendices

Comparison of breast density measured by dual energy x-ray Absorptiometry (DXA) with mammographic density among adult women in Hawaii

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ABSTRACT
While use of mammography is limited, due to concerns related to radiation exposure, Dual Energy X-ray Absorptiometry (DXA), commonly available in medical care settings, is characterized by low radiation exposure. In the current paper, we compared breast density measured by DXA with mammographic density in 101 adult women who had a screening mammogram during the last 2 years. DXA scans of both breasts were taken using a clinical DXA system calibrated to measure breast density. The total projected breast area was manually delineated on each image and percent fibroglandular volume density (%FGV), absolute fibroglandular volume, total breast area and volume were computed. After digitizing mammographic films, total breast area, dense area, and percent density (PD) were estimated using computer-assisted mammographic density assessment. Both DXA and mammographic measures showed high correlations between left and right breasts ranging from 0.85 to 0.98 (p <0.0001). Mean %FGV was 38.8±14.3%, and mean percent density was 31.9±18.2% for craniocaudal views and 28.3±16.2% for mediolateral views. The correlation between the two measures was 0.76 for both views (p <0.0001). Associations with common risk factors showed similar patterns for DXA and mammographic densities; in particular, the inverse associations with BMI and age at menarche were evident for both methods. Multilinear regression with stepwise selection indicated an explained variance of 0.56 for %FGV alone and of 0.58 for %FGV plus number of children. Despite some differences in methodology, the current comparison suggests that DXA may provide a low-radiation option in evaluating breast density.

Key words: DXA, mammographic density, mammogram, breast, adult, risk factors

INTRODUCTION

Mammographic density, the distribution of fat, connective, and epithelial tissues in the female breast, is strongly associated with breast cancer and has been used as a biomarker for breast cancer risk among adult women [1, 2]. However, use of X-ray based mammography is limited due to concerns related to radiation exposure. In contrast, Dual Energy X-ray Absorptiometry (DXA) is characterized by low radiation exposure and commonly available in medical care settings. As shown among 17 women, a commercial DXA device, when it is calibrated to measure breast density, provides a precise measure of breast composition in comparison with mammography [3]. This suggests that DXA may provide an additional tool for evaluating breast cancer risk with minimal radiation exposure. For now, DXA breast imaging serves as a research tool to investigate breast development in girls and young women. However, in the future it may play a role in individualized risk prediction [4]. In the current paper, we compared breast density measured by DXA and by regular mammography in women who took part in a study that included mothers and their adolescent daughters 10-16 years of age. An additional objective of this report was to explore a prediction model for mammographic density using the DXA measure in combination with demographic, anthropometric, and reproductive information.

MATERIALS AND METHODS

Study design and procedure. The current analysis was conducted as part of a study that measured breast density and body-fat composition in adult women and their daughters using DXA. The project was approved by the Committee on Human Studies at the University of Hawaii and the Institutional Review Board of Kaiser Permanente (KP) Hawaii. We recruited women aged 30 years and older who had received a normal mammogram (defined as BIRADS categories 1 through 3) during the last 2 years and their daughters aged 10-16 years through KP Hawaii, a large health maintenance organization. We mailed 3,915 invitation letters to women and to girls in the respective age ranges over the course of 11 months. Potential participants were selected from the membership data base according to the age and mammographic criteria. From the 304 respondents, we excluded mothers who had no mammograms, a previous history of breast cancer or surgery, an abnormal mammogram, a previous biopsy, breast implants, or chronic health conditions that interfered with study participation. We excluded girls without breast development and mother-daughter pairs who were not biologically related or did not reside on Oahu. A few daughters were recruited though KP whose biological mother was not a KP member.

Of the 138 eligible mother-daughter pairs, 102 pairs completed the study visit. Prior to DXA scans, all mothers signed informed consent, answered a demographic questionnaire, and completed height and weight measurements in duplicate. The six women whose screening mammograms were not performed at KP signed a mammogram release authorization form for the respective clinics. In the questionnaire, participants reported ethnicity and reproductive factors, i.e., age at menarche, age at first live birth, number of children, menopausal hormone use, and the most recent menstrual period. Women whose last menstrual period was >1 year ago were classified as postmenopausal. Body mass index (BMI) was calculated using the height and weight and classified as normal (18.5 - <25), overweight (25 - <30) and obese ($\geq$30 kg/m^2).

DXA data collection. At the exam, a urine test excluded pregnancy in all participants. We performed DXA scans of both breast, as well as of the whole body, using the research scan protocol and software version 10.1 on a GE Lunar Prodigy Bone Densitometer (GE Healthcare).

Prodigy utilizes an ultra low radiation and cadmium-zinc-telluride detector to convert X-rays into an electronic signal without the intermediate conversion to light. We used a custom thickness-step phantom made of reference materials to recalibrate the DXA device. Details of this phantom and mathematical equations to compute breast density and thickness were described elsewhere [5, 6]. After changing into a hospital gown, breast scans were taken on both breasts in the decubitus mediolateral position with the nipple positioned in a true lateral profile. A duplicate breast scan of the left breast was performed on a random 10% of subjects for quality control.

A low energy and high energy attenuation image was saved for each scan using the options available from GE Lunar for the research scan mode. These images were analyzed by the University of California at San Francisco using a Breast Density Workstation. The total projected breast area was manually delineated on each image by the same operator (Figure 1). From the bottom of the breast, the delineation followed the thoracic cage, then the pectoral muscle to continuously reach the exterior of the breast. Finally the external line was delineated. To calculate DXA density, a two-compartment model of adipose and fibroglandular tissue was used [7]. Scans of a calibrated phantom with known composition and thicknesses allowed the calculation of calibration curves. We computed total breast area, breast volume, absolute fibroglandular volume (FGV), and percent FGV (%FGV).

For quality control, a calibrated phantom was scanned over 8 months, once every day if participants were scheduled, and once a week if no participant was scheduled. The phantom varied in thickness (2, 10, and 20 cm) and contained three %FGV values of 28%, 65%, and 100%. The phantom precision values ranged from 1.9% (10 cm in thickness and 65% composition) to 5.4% (2 cm in thickness and 65% composition). For the repeated breast scans, a root mean square standard deviation of 2.5 and a correlation of 0.975 were achieved.

Mammographic data. Craniocaudal (CC) and mediolateral (ML) views of screening mammograms taken within the last 2 years were scanned with a Kodak LS85 Film Digitizer for 101 women; one film could not be located. Ninety-five women had film mammography at KP, and 6 women had digital mammography at non-KP clinics and hospitals. All personal identifiers were removed from the scanned images. One of the authors (GM) performed computer-assisted density assessment using the Cumulus package [8, 9]; all mammograms for one woman were assessed during the same session. Using this interactive method, the reader selects a threshold value (gray scale on the screen) that best distinguishes the breast from the dark background and another threshold value, the gray value that best identifies the edges of the mammographically dense areas within the breast outline. The number of pixels in the two areas is then measured by the computer. The mammographic measures for our analysis included the total breast area and the dense area of the breast; percent density (PD) was calculated as the ratio of the two. In a sample of 49 duplicate mammographic readings, the correlations were 0.997 for the size of the total breast area and 0.959 for the dense breast area resulting in a coefficient of 0.955 for PD.

Statistical Analysis. All statistical analyses were performed using the SAS statistical software package version 9.2 (SAS Institute, Inc., Cary, NC). Correlations between right and left breasts measures were computed for both DXA and mammographic measures. The correlation between the two methods was evaluated using their corresponding measures, i.e., total area (cm^2) by DXA and total area (cm^2) by mammography, FGV (cm^3) and dense area (cm^2), and %FGV and PD. Because of the small sample size, subjects were grouped into 3 major ethnic categories: Caucasian, Asian (Japanese, Chinese, Filipino, Korean, and Other Asian), and Other (Hawaiian

and other Pacific Islanders, Black, Native American, Hispanic, and Other). Correlation coefficients and analysis of variance were used to detect statistically significant differences in demographic characteristics by ethnic category. Mean DXA and mammographic measures were calculated by ethnicity and relevant risk factors, i.e., age, BMI, age at menarche, age at first live birth, number of children, menopausal status, and menopausal hormone use. To develop a prediction formula, we performed multilinear regression using the stepwise selection method with PD as dependent variable and %FGV and all covariates as independent variables. An α-level of 0.15 was used to select independent predictors for inclusion in the final model.

RESULTS

The ethnic background of the study population was 31% Caucasian, 46% Asian, and 23% Other (Table 1). With one exception (age of 64 years), the ages ranged between 39 and 58 years. The three ethnic groups differed in BMI, age at menarche, and age at first live birth. The mean BMI was highest in the Other group ($p = 0.01$), while the mean age at menarche was lowest among Asians and highest among Others ($p < 0.001$). Age at first live birth was higher in Asian and Caucasian than in Other women ($p = 0.01$). Mammographic density, DXA measures, menopausal status, number of children, and menopausal hormone use did not differ significantly by ethnicity although PD for the CC view was marginally significant ($p = 0.08$).

When comparing left and right breasts, both DXA and mammographic measures showed high correlations (Table 2) ranging from 0.85 to 0.98 ($p < 0.0001$) with a stronger association for CC than ML views. In addition, correlation coefficients were higher for DXA measures (0.96 to 0.98) than mammographic measures (0.85 to 0.95).

For the overall population, mean %FGV was 38.8±14.3, and mean PD was 31.9±18.2 for CC and 28.3±16.2 for ML views. PD in CC and ML views were strongly correlated with %FGV (Figure 2). The associations between DXA and mammographic measures were >0.80 for the total area and 0.76 for PD ($p < 0.0001$ for both). However, the correlation coefficients between DXA FGV (cm^3) and dense area (cm^2), which possessed different dimensional characteristics, were 0.30 to 0.36 for CC and ML views ($p < 0.01$ and < 0.001, respectively).

In evaluating the relation of DXA and mammographic measures with characteristics that have previously been found to be associated with breast density, we observed highly significant inverse associations of BMI with %FGV and PD (Table 3). The respective differences between normal weight and obese women were 22% and 25% ($p < 0.001$ for both). As to reproductive characteristics, we observed non-significant, higher %FGV and PD in menopausal hormone users than non-users and significantly higher %FGV and PD among pre- than postmenopausal women. Age at menarche was positively associated with %FGV and PD ($p < 0.0001$ and 0.04, respectively). Age and ethnicity did not show significant associations. DXA and mammographic density were non-significantly lower with more children ($p = 0.47$ and 0.09, respectively).

Multilinear regression with stepwise selection indicated an adjusted r^2 of 0.56 for %FGV to PD both for the CC and the MLO views, and an adjusted r^2 of 0.58 when the number of children was added. Other demographic and reproductive variables including menopausal hormone use did not contribute to the model. We also included a time variable describing the number of days between mammography and DXA. The mean time difference was 5.6 ± 4.2 months and inclusion of the time variable did not modify the regression estimates.

DISCUSSION

The current study compared the breast density of adult women calculated from DXA images with mammographic density from screening mammograms. We observed moderate to high correlations between the two methods: 0.83-0.89 for total area, 0.30-0.36 for FGV and dense area and 0.76 for %FGV and PD. Moreover, the associations with demographic, BMI, and reproductive characteristics showed very similar patterns for DXA and mammographic density, in particular, the strong inverse association of PD with BMI was evident for both methods. In comparison with a previous report that computed a correlation of 0.52 between PD and %FGV [3], the current analysis evaluated DXA in a larger sample of women and found a stronger association between DXA and mammographic measures. Correlations between left and right measures were comparable in both DXA and mammography, and the linear association between DXA and mammographic measures were also strong. The correlation between FGV and dense area was considerably lower. This may be in part due to the breast compression used for mammography but not for DXA, but primarily it is due to the 3-dimensonial nature of FGV in contrast to the 2-dimensional mammograms. Other volumetric density methods suggest that percent density may be lower when assessed as a volumetric measure than as the 2-dimensional percentage of the breast area [10, 11]. As apparent from Figure 2, the lowest DXA density was approximately 20% while several women had mammographic density values below 20%. The reason for this is that DXA measures the breast as compartments of fibroglandular tissue and fat. In DXA, fat is used as a reference since the water content of adipose tissue changes and is not a stable reference. Thus, the breast does not get leaner than about 20%FGV, the fraction of water in adipose tissue. A similar result was presented by a report using magnetic resonance images (MRI) volume of fat and water to measure breast density [12].

Similar to DXA, a number of different methods are currently under investigation to examine breast density without radiation. Boyd et al [12] evaluated the use of MRI in 400 mother-daughter pairs and found a strong correlation ($r = 0.85$, $p <0.0001$) between percent breast water (as surrogate for fibroglandular-tissue level) calculated from MRI and mammographic density among 100 mothers. The same study also evaluated the use in young women 15-30 years and compared the results with density measures of their mothers. Investigators in Minnesota evaluated the use of ultrasound tomography images to assess breast density and found positive associations with mammographic density [13, 14]. Both MRI and ultrasound tomography are non-radiation methods and require no breast compression. One major advantage of DXA as compared to the other methods, both mammography and other novel approaches, is that it is widely available in clinics and hospitals, more than 30,000 systems worldwide, and relatively inexpensive. Unlike mammography, it provides three-dimensional data for density calculation, does not require a subjective interpretation of results [7], and has been explored as a research tool among young girls during pubertal development [6]. Furthermore, DXA provides additional information on whole body composition that may be useful in assessing additional breast cancer risk factors.

Some of the limitations of our analysis include the narrow age range of subjects, the exclusion of nulliparous women, and the lack of data in breast cancer cases. The combination of the restricted age range, the inclusion of only parous women, the high proportion of women who reported more than one ethnic background, and the small sample size are probably responsible for a lack of association of age and ethnicity with breast density in this study. Thus, our results need to be replicated in broader populations and should be interpreted with caution. Despite the great stability of mammographic density over time [15], breast density may change, especially during the menopausal transition or due to menopausal hormone therapy [16, 17]. Therefore, it

was not ideal that we compared DXA and mammogram images taken at different times, but the time difference was not a significant predictor of PD in a multiple regression model with %FGV as an independent variable. Based on our previous study among premenopausal women [18], the mean annual change in PD is less than 2%; for 94 out of 101 women in the current analysis the time difference was less than one year. Thus, the differences should be very small. A strength of our study is the excellent quality control result. Duplicate readings of a subset of mammograms showed high reproducibility with a coefficient of 0.955 for PD. The calibration data and the repeated breast scans indicated high precision of the DXA data. Our multiethnic population strengthens the generalization of our results.

Our findings suggest that DXA has potential to provide a low-radiation option for evaluating breast density and may be useful as part of breast cancer risk prediction although it is unlikely to play a role in the detection of abnormalities [4, 19]. Its current use is restricted to being a research tool, but in the future, DXA imaging may allow the identification of high-risk girls and young women and make it possible to offer them early detection approaches and/or interventions to reduce breast cancer risk. The method has advantages of relative low cost, broad availability, and simultaneous provision body composition data. However, we need more imaging information from younger women and from breast cancer cases to evaluate the method.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

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Table 1. Characteristics of study participants by ethnicity

Ethnic category	Caucasian	Asian	Other	All	p value[a]
N	32	47	23	102	--
Age (years)	48.0±4.4	48.3±5.3	46.2±3.8	47.7±4.8	0.19
BMI (kg/m^2)	26.6±5.8	26.5±5.6	31.1±5.8	27.5±5.9	<0.01
Age at menarche (years)	12.9±1.6	11.9±1.2	13.3±1.9	12.5±1.6	<0.001
Age at first live birth (years)	30.0±5.4	30.1±5.8	26.0±5.4	29.1±5.8	0.01
Number of children (N)	2.5±1.0	2.3±0.8	2.9±1.3	2.5±1.0	0.07
Postmenopausal status (N)[b]	9	12	7	28	0.89
Menopausal hormone use (N)	5	13	4	21	0.38
Overweight/Obese (N)[c]	9/8	17/8	7/12	33/28	0.02
DXA total volume (cm^3)	819.5±521.0	746.6±448.2	1005.7±535.5	827.9±497.6	0.12
total area (cm^2)	95.6±43.4	85.2±35.5	103.3±38.2	92.5±39.0	0.16
FGV (cm^3)	275.9±103.7	259.9±112.8	298.6±102.3	273.7±107.7	0.37
%FGV	40.4±14.0	40.1±15.3	33.9±11.8	38.8±14.3	0.18
Mammographic PD CC view	30.9±19.6	35.8±17.4[d]	25.5±16.2	31.9±18.2	0.08
ML view	29.8±18.4	29.9±15.6[d]	23.3±13.5	28.3±16.2	0.23

[a]p value based on analysis of variance or chi-square test
[b]Last menstrual period more than one year ago; one woman had missing data.
[c]Overweight: BMI 25-30 kg/m^2; Obese: BMI ≥30 kg/m^2
[d]N=46; one film could not be located.

Table 2. Summary of DXA and mammographic measures for left and right breasts

			Left[a]	Right[a]	r^b	p
DXA	Total volume (cm^3)		834.6±508.3	821.2±490.5	0.98	<0.0001
(N=102)	Total area (cm^2)		93.2±39.0	91.8±39.7	0.97	<0.0001
	FGV (cm^3)		275.0±109.8	272.3±108.1	0.96	<0.0001
	%FGV		38.7±14.3	38.8±14.5	0.97	<0.0001
Mammography	Total area (cm^2)	CC view	122.7±57.6	112.8±52.7	0.90	<0.0001
(N=101)		ML view	130.8±53.6	125.8±54.1	0.95	<0.0001
	Dense area (cm^2)	CC view	34.2±22.3	31.1±18.1	0.88	<0.0001
		ML view	32.3±20.2	32.7±21.7	0.85	<0.0001
	PD (%)	CC view	31.7±18.4	32.2±18.8	0.90	<0.0001
		ML view	28.1±16.9	28.6±16.7	0.87	<0.0001
DXA and	Total area (CC view)				0.83	<0.0001
mammography	Total area (ML view)				0.89	<0.0001
	FGV and dense area (CC view)				0.30	<0.01
	FGV and dense area (ML view)				0.36	<0.001
	%FGV and mammographic PD (CC view)				0.76	<0.0001
	%FGV and mammographic PD (ML view)				0.76	<0.0001

[a]Mean and standard deviations
[b]Spearman rank order correlation coefficient.

Table 3. DXA and mammographic measures by breast cancer risk factors

Risk factor	Category	N	FGV (%)[a]	p[b]	N	PD (%)[a]	p[b]
Age (years)	<45	34	41.1±16.7		33	32.0±18.0	
	45-49	37	36.9±11.7		37	33.9±17.5	
	≥50	31	38.5±14.3	0.45	31	29.5±19.4	0.59
Ethnicity	Caucasian	32	40.4±14.0		32	30.9±19.6	
	Asian	47	40.1±15.3		46	35.8±17.4	
	Other	23	33.9±11.8	0.18	23	25.5±16.2	0.08
Body mass index (kg/m^2)	18.5-<25	41	48.8±15.0		41	42.7±15.8	
	25-<30	33	36.3±8.1		33	30.0±16.8	
	≥30	28	27.1±7.0	<0.0001	27	17.8±12.0	<0.0001
Age at menarche (years)	<13	60	34.5±10.5		59	28.8±16.7	
	≥13	42	44.9±16.6	<0.001	42	36.2±19.4	0.04
Age at first live birth (years)	<30	60	38.4±15.2		59	29.2±18.2	
	≥30	42	39.2±13.3	0.76	42	34.9±17.9	0.11
Number of children (N)	1-2	59	39.7±15.8		59	34.5±18.7	
	≥3	43	37.6±11.8	0.47	42	28.3±17.0	0.09
Menopausal status[c]	Pre	73	40.5±14.7		72	34.6±17.2	
	Post	28	34.3±12.5	0.05	28	25.8±19.3	0.03
Menopausal hormone use	Yes	22	38.6±13.4		22	29.9±18.2	
	No	80	38.8±14.6	0.95	79	32.5±18.2	0.56

[a]Mean and standard deviations of left and right breast measures (CC view for mammographic density)
[b]Calculated by ANOVA as the trend across categories
[c]One women had missing data; N=101

Figure 1. Breast images by DXA (left) and mammography (right)
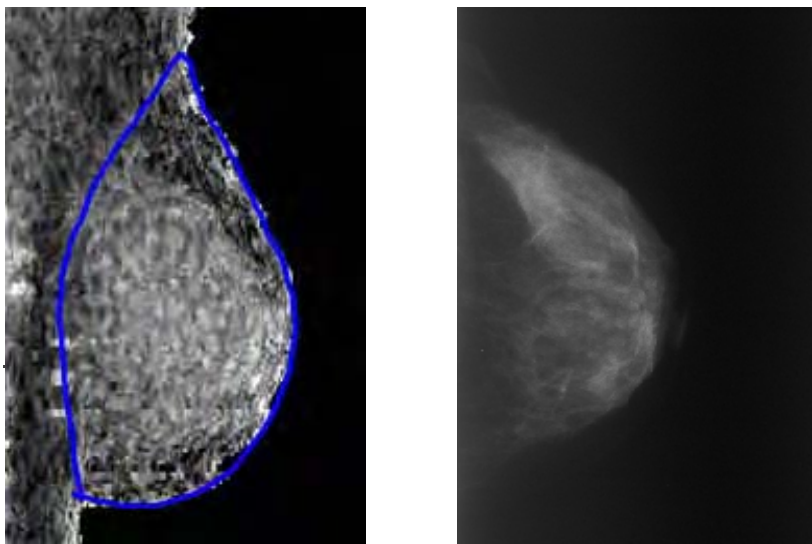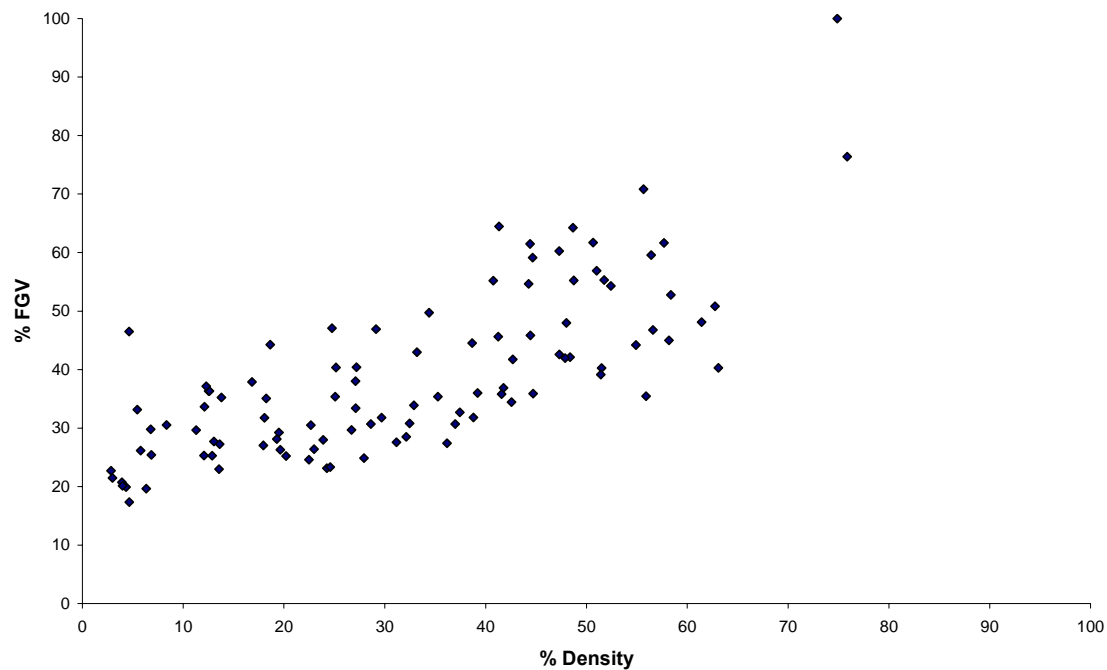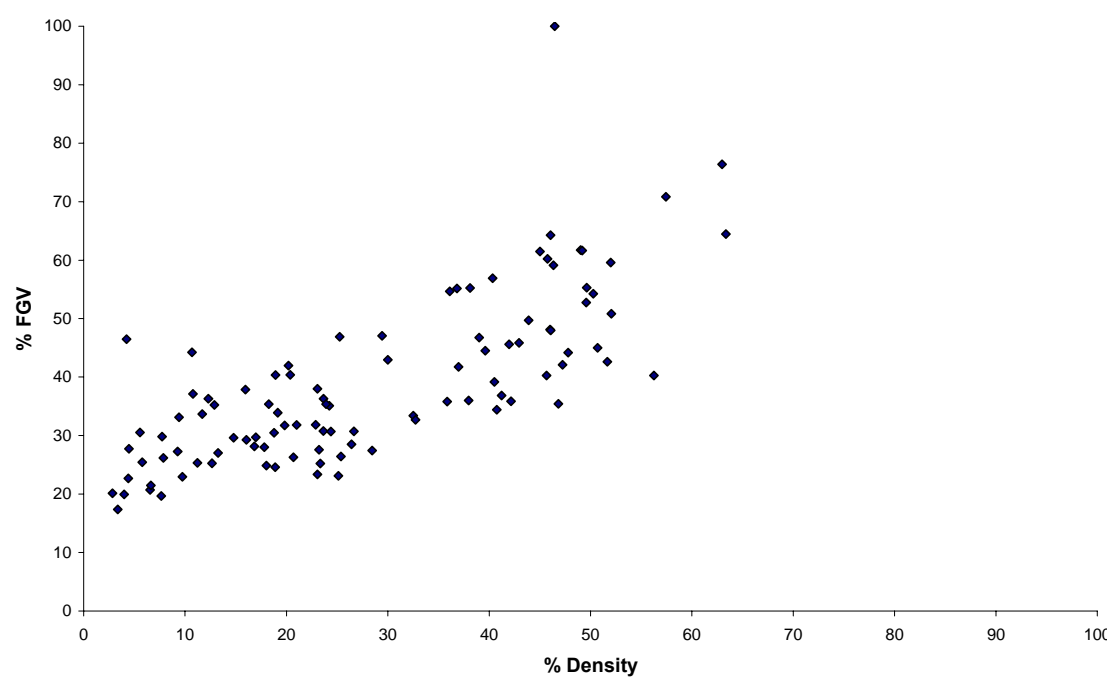
A. Fatty breast
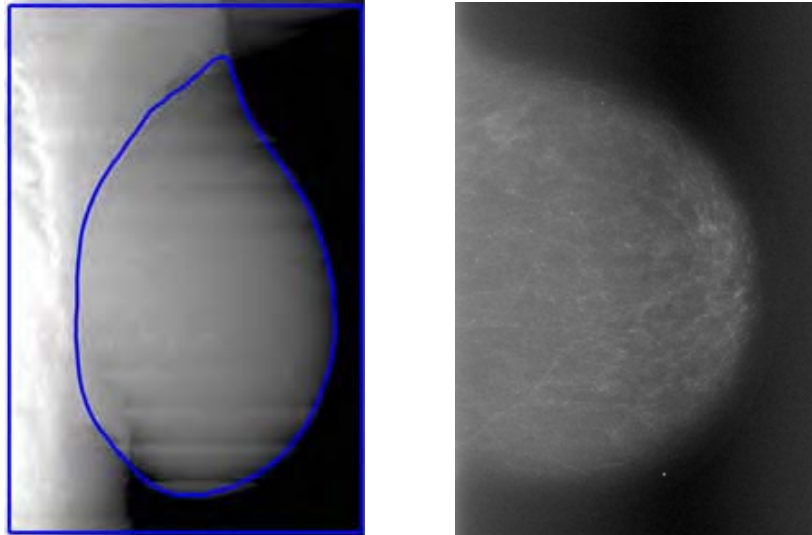
B. Dense breast

Figure 2. Correlation of breast densities measured by DXA and mammography

A. Craniocaudal View

B. Mediolateral View

Body fat and menarche are associated with breast density in multiethnic adolescent girls [1,2]

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Abstract

Objectives: The aim of this paper is to examine DXA, as a novel method to assess breast density in relation to growth and maturation of multiethnic adolescent girls. **Methods**: We recruited 113 girls and their mothers from Kaiser Permanente Hawaii. Ethnicity and age at menarche were determined by interview. Weight and height were measured, and body mass index (BMI) and BMI Z-scores were calculated. Tanner breast and pubic hair stages were assessed visually. DXA scans of the total body provided body fat measures and DXA scans of both breasts were performed using a GE Lunar Prodigy DXA with special calibration for the breast measures. Using manual delineation, total breast area, volume, absolute and percent fibroglandular volume (%FGV) were computed. Correlation and stepwise regression were conducted to evaluate the relationship between measures. **Results**: A linear relationship of breast area, volume, and FGV with Tanner breast stages was found ($p<0.001$). BMI was inversely associated with %FGV ($p<0.0001$) while breast area, volume, and FGV showed a positive association ($p<0.0001$). In a stepwise regression model that explained 80% of the variability, significant predictors of %FGV were, in order, percent body fat, achieved menarche, BMI z-score, Tanner breast stage, and "Other" ethnic group. A similar model, that did not include menarche and did include age, predicted 77% of the variability in breast density. **Conclusions**: Thus, breast density in adolescent girls was inversely associated with measures of adiposity and was positively associated with measures of maturation.
Key words: DXA, breast density, adolescent, menarche

INTRODUCTION

Mammographic density, the distribution of fat, connective, and epithelial tissues in the female breast, is strongly associated with breast cancer and has been used as a biomarker for breast cancer risk among adult women (Boyd et al., 2007; Cormack VA and dos Santos Silva I 2006). However, use of X-ray based mammography is limited due to concerns related to radiation exposure. In contrast, Dual Energy X-ray Absorptiometry (DXA) is commonly available in medical care settings and has very low radiation (30 µSV for whole body and 15 µSV for a breast scan) (Laskey 1996). As shown in a small sample of women (n=18), a commercial DXA device. calibrated to measure breast density, provides a precise measure of breast composition, in comparison with mammography (Shepherd et al., 2006). This suggests that DXA may provide an additional tool for evaluating breast cancer risk with minimal radiation exposure, allowing for more or earlier breast screening. Some studies have demonstrated that the addition of body size variables to the Gail model of breast cancer risk improve the prediction of breast cancer risk (Chen et al., 2006).

Obesity is a risk factor for postmenopausal breast cancer (Pischon et al., 2008; Renehan et al., 2008). The prevailing hypothesis is that, after menopause, adipose tissue becomes a major source of endogenous estrogens, which increases breast cancer risk by converting adrenal steroids to estrogens (Key et al., 2003; Pischon et al., 2008; Renehan et al., 2008). In addition, high insulin levels, resulting from obesity, inhibit secretion of sex hormone-binding globulin (Pischon et al., 2008), further contributing to elevated estrogen levels. However, obesity during child or adolescent growth may also affect breast tissue and, possibly more profoundly. For example, breast density in adulthood appears to be inversely associated with adolescent weight, body mass index (BMI), and adiposity, and to be positively related to adolescent height. (Ahlgren et al., 2004; McCormack et al., 2003; Sellers et al., 2007) Adolescence is believed to be a time of great breast susceptibility to carcinogens because breast cells are only fully matured after a first pregnancy (Russo and Russo 2000). Development of methods to examine breast tissue development during adolescence may allow discovery of important breast cancer risk factors, and their prevention. Our group has demonstrated feasibility of a DXA method to evaluate density of breast tissue in adolescent girls (Shepherd et al., 2008). The objectives of this paper are to examine the novel DXA breast density measurement in relation to various measures of growth and pubertal maturation in multiethnic girls that are 10-16 years old.

METHODS

Study design and procedure. The current analysis was done as part of a study that measured Tanner stages of pubertal development, breast density, and body composition in adult women and their adolescent daughters. The project was approved by the Committee on Human Studies of the University of Hawaii and the Institutional Review Board of Kaiser Permanente Hawaii (KPH). We recruited women aged 30 y and older, who had received a normal mammogram (defined as Mammogram Breast Imaging Reporting and Data System, BIRADS, Score of 1 - 3) during the last two years, and their daughters (aged 10-16 years) through KPH, a large health maintenance organization. We mailed 3,915 invitation letters over the course of 11 months to potential participants who were selected from the KPH membership electronic data base according to the maternal age and mammographic eligibility criteria. From the 304 mail respondents, we excluded mothers who had a previous history of breast cancer or surgery, an abnormal mammogram, a previous biopsy, breast implants, or chronic health conditions that interfered with study participation. Girls (daughters) were eligible if they were 10-16 years old

and reported some breast development. Mother-daughter pairs that were not biologically related, or who did not reside on the island of Oahu, were excluded. Of the 138 eligible mother-daughter pairs, 101 pairs completed the study visit. Because 12 mothers had two eligible daughters, the study included 113 girls. There was missing whole body DXA data for one girl, so data involving % body fat and TPFR are presented for n=112.

Prior to DXA scans, all mothers signed informed consent and all daughters signed informed assent. All participants answered a questionnaire that asked for the occurrence of menarche and the age of occurrence of menarche, the percentages of all ethnic backgrounds that applied to their parents, and for one primary ethnicity with which they most identified; the latter ethnic definition is used in this paper. Tanner breast and pubic hair stages were determined by clinical visual inspection of the breast and of the pubic hair development, by one trained research staff. BMI was determined from measured height and weight. Subsequently, we obtained BMI Z score and BMI-for-age-percentile according to CDC reference data (Centers for Disease Control and Prevention (CDC) 2006).

DXA data collection. At the study exam, a urine test excluded pregnancy in all participants. We performed DXA scans of both breasts according to our own protocol (Shepherd et al., 2006; Shepherd et al., 2008) and standard whole body DXA scan using a GE Lunar Prodigy Bone Densitometer (GE Healthcare). For the breast scan, we followed the research scan protocol (software version 10.1) using a custom thickness-step phantom made of reference materials to recalibrate the DXA device. Details of this phantom, and mathematical equations to compute breast density and thickness, are described elsewhere (Shepherd et al., 2005; Shepherd et al., 2008). After changing into a hospital gown, breast scans were taken on both breasts in the decubitus mediolateral position, with the nipple positioned in a true lateral profile. A duplicate breast scan of the left breast was done on a random 10% of subjects for quality control. A low and a high energy attenuation image were saved for each scan using the options available from GE Lunar for the research scan mode. The images were analyzed by the University of California at San Francisco using software written in Matlab (Mathworks, Natick, MA).

Total projected breast area was manually delineated on each image by the same operator (Fig. 1). From the bottom of the breast, the delineation followed the thoracic cage, then the pectoral muscle, to the exterior of the breast. Finally, the external line of breast tissue was delineated. To calculate DXA breast density, a two-compartment model of adipose and fibro-glandular tissue was used (Shepherd et al., 2002). Scans of a calibrated phantom with known composition and thicknesses allowed the calculation of calibration curves. Total breast area, breast volume, absolute fibro-glandular volume (FGV), and percent fibro-glandular volume density (%FGV) were computed.

For quality control, a calibrated phantom was scanned over a period of eight months, once every day if participants were scheduled, and once a week if no participant was scheduled. The phantom varied in thickness (2, 10, and 20 cm) and contained three %FGV values of 28%, 65%, and 100%, respectively. The phantom precision values ranged from 1.9% (10 cm in thickness and 65% composition) to 5.4% (2 cm in thickness and 65% composition). For the repeated breast scans, a root mean square standard deviation of 2.5 and a correlation of 0.975, were achieved.

For the whole body scan, performed according to standard procedures and calibration, we determined percent total body fat, trunk body fat (g), arm fat (g), and leg fat (g). As a measure of

central or upper body fat distribution, trunk-to-periphery fat ratio (TPFR) was calculated as the ratio of trunk fat over arm fat plus leg fat (Novotny R et al., 2006).

Statistical Analysis. Statistical analyses were done using SAS statistical software version 9.2 (SAS Institute, Inc., Cary, NC). Results were reported as means and standard deviation (SD); an α-level of 0.05 was considered significant. Because of the small sample size, subjects were classified into one of three ethnic categories based on self-reported primary ethnicity: White, Asian (Japanese, Chinese, Filipino, Korean, and Other Asian), and Other (Hawaiian, Pacific Islanders, Black, Native American, Hispanic, and Other). We examined plots and correlations among breast measures and age, Tanner stages, menarcheal groups, and body fat measures. Correlation coefficients and analysis of variance were applied to detect differences in characteristics by ethnic group, body fat categories, and DXA breast measures. We performed a stepwise regression analysis with an α-level of 0.15 to predict %FGV, using the following independent variables: ethnicity (Asian, Other and White as reference group), age, achieved menarche (yes, no), Tanner pubic hair stage, Tanner breast stage, weight, height, BMI z-score, and % total body fat.

RESULTS
The investigation included 113 girls of White (n=35), Asian (n=41), and "Other" ethnicity (n=37). Thirteen percent (15/113) of girls were considered overweight according to CDC percentiles and another 15/113 (13%) were obese; only two girls were below the fifth percentile. The mean age at menarche for the 80 out of 113 girls who had reached menarche was 11.7 ± 1.1 y (range: 9-14 y). Weight and height, but not BMI, differed significantly by ethnicity (data not shown). DXA % total body fat did not differ by ethnic group (unadjusted for other variables), though TPFR did, with both Asian (estimate $0.149 \pm$ SE 0.036, p<0.0001; or 15% higher) and "Other" (estimate $0.076 + 0.037$, p=0.043; or 8% higher) ethnic groups showing higher TPFR than Whites.

Girls were at the same stage for breast and pubic hair assessments (synchronous) in 44 of 111 girls (40%) of those who completed both assessments. Girls were in a higher breast stage than pubic hair stage in 51 of 111 cases (46%) and in higher pubic hair than breast stage in 17 or 111 cases (15%). White girls were synchronous in Tanner stages in 11 of 35 (31%) of cases, Asian 17 of 39 (44%) of cases and girls of "Other" ethnicity in 15 of 37 (41%) of cases. White girls were in a higher pubic hair stage than breast stage in nine of 35 cases (26%), Asian girls in two of 39 cases (5%) and "Other" girls in six of 37 cases (16%); these ethnic differences were significant (OR= 0.16 (C= 0.031-0,783, p= 0.03). The remaining 15 of 35 (43%) White girls were in higher breast than pubic hair stage, as were 20 of 39 (51%) of Asian girls, and for 16 of 37 (43%) "Other" girls; there was no significant ethnic difference.

A high resolution image of an Asian girl in Tanner breast stage 3, who is 12.6 y old and at the 34th percentile of BMI for age, is shown in Fig. 1. We observed significant differences in breast area, breast volume, and absolute FGV with Tanner stages (Table 1), both for breast and pubic hair. On the other hand, %FGV increased from Tanner 1 and 2 to Tanner 4, but declined from Tanner 4 to Tanner 5. The strongest correlation with %FGV was % body fat at -0.80 (Table 1). Breast area, breast volume, and absolute FGV were positively related to measures of body fat (Fig. 2A), when girls were stratified into quartiles of body fat measured as BMI, BMI Z score, % body fat, or TPFR. On the other hand, %FGV showed an inverse association with all measures of body fat (Fig. 2B). As illustrated in Figs. 3A and 3B, %FGV decreased by tertile of

body fat in all groups, but did not differ by ethnicity. %FGV was higher with attainment of menarche and varied to a greater degree before menarche than after menarche.

In a stepwise regression model, significant predictors of %FGV were, in order, percent body fat, menarche, BMI z-score, Tanner breast stage, and "Other" ethnic group (Table 2A). The model explained 80% of the variability in %FGV. Those who achieved menarche had 11.5% more %FGV (breast density). A stepwise regression model without menarche explained 77% of the variance in %FGV (Table 2B); the strongest predictor was again percent body fat, but age was now selected in place of menarche, followed by Tanner breast stage, BMI z-score and then "Other" ethnic group.

DISCUSSION

Breast density, as assessed by %FGV from a DXA image, was inversely associated with body fat measures among multiethnic adolescent girls, as has been found in studies using mammograms among adult women (Samimi et al., 2008). While 67% of the variability in %FGV was explained by percent body fat alone, having achieved menarche explained an additional 10% of variability, more than the eight percent that age contributed in a model without menarcheal status. Interestingly, %FGV increased up to Tanner breast stage 4, but was lower for Tanner breast stage 5. This suggests that this final maturation stage consisted of growth in breast fat rather than in fibro-glandular tissue. A similar finding was described in our pilot study with 18 girls (Shepherd et al., 2008) .

Similar to our study, Himes et al. found that Tanner pubic hair stage was not associated with DXA fat mass though Tanner breast stage was strongly predictive of DXA fat mass among African American girls (Himes et al., 2004). In that study, the girls' fat mass increased 90% between Tanner breast stage 1 and 2. In our study, Tanner pubic hair stage was not selected in stepwise models predicting breast density (%FGV), though Tanner breast stage consistently was. Like Himes et al., we found that total body fat mass increased with Tanner breast stage (Table 1). However, in our study it increased after Tanner breast stage 3 (when menarche tends to occur), among our predominantly Asian and White ethnic groups. In our population, we observed little difference in breast density (%FGV) by ethnic group across tertiles of body fat (Fig. 3A). In Himes' study (Himes et al., 2004), risk for overweight, according to age-adjusted BMI percentiles, was more than six times greater among those that had begun breast development. While still a good indicator of the onset of fat gain, in our population the fat gain occurred in later Tanner breast stages (3 and higher).

Variation in pattern of breast growth by ethnicity could be a result of hormonal influences that may be meaningful for breast cancer risk. Boyd et al (Boyd et al., 2009) showed that sex hormone-binding globulin (SHBG) and growth hormone are positively associated with total breast water, a measure of the proportion of the breast that is fibro-glandular tissue, analogous to our %FGV measure. Free testosterone was inversely associated with total breast water. Ethnic differences in hormonal levels during puberty could influence breast growth, resulting in varied breast densities as previously described for women in Hawaii (Maskarinec et al., 2007).

Since breast development is stimulated chiefly by estrogens (Styne D 2001), obesity may have an influence on risk for future breast cancer. Obesity may advance breast maturation through the aromatization of androgens into estrogens in adipose tissue. Additionally, the relatively lower levels of SHBG among obese individuals, makes larger proportions of hormones, such as testosterone and estradiol, available for biological activity (Mendel 1989). Pubic hair development, on the other hand, is determined by adrenal and ovarian androgens.

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Also, further examination of nipple and areolar development may help discriminate the nature of breast development (Biro F et al., 1992).

Biro et al described two pathways of pubertal maturation - the thelarche pathway (initiated by areolar and breast development) and the adrenarche pathway (initiated by pubic hair development) (Biro F et al., 1992). The former was more strongly associated with overweight (including higher waist to height ratio, analogous to our TPFR), and with earlier menarche, in a longitudinal study of White girls (Biro et al., 2008). Asynchronous maturation between breast and pubic hair was typically characterized by the thelarche pathway. Identifying the pathway of onset of puberty may increase sensitivity of risk models, which is associated with high levels of adipocyte-specific aromatase and other relevant biochemical factors, such as leptin (Garcia-Mayor R et al., 1997).

With our cross-sectional study design we were not able to assess onset of menarche or onset of Tanner stages. Although Tanner reference data from White girls suggest that breast maturation (stages) occur first and that breast maturation lasts longer than pubic hair maturation (stages), we compared concordance of breast and pubic hair stages. Our study shows that more Asian girls were synchronous in breast and pubic hair maturation than White girls. However, asynchronous Asian girls were more likely to have higher breast than pubic hair stages (51%) than were White girls (43%), suggesting a more thelarcheal pathway of maturation among these girls. Girls of "Other" ethnicity were intermediate as would be expected. These pubertal synchrony data, showing the Asian girls with asynchrony to be more thelarcheal than Whites, and the data showing that Asian girls have higher TPFR than Whites, both demonstrate greater central obesity of Asians compared to Whites, even though BMI was not greater, as we have found before (Novotny R et al., 2006).

The major limitation of our analysis is the lack of longitudinal information for the same girls that would allow us to examine changes in breast density with maturation. Strengths of our study include the excellent quality control results for the DXA measures and the multiethnic population, which strengthens generalization of our results. Since risk modeling has suggested that breast maturational status within overweight girls may be associated differentially with health risks, (e.g., such as adult obesity, breast cancer and high blood pressure (Himes JH 2002), more longitudinal studies are needed to disentangle the causality of these associations among ethnic groups. Our study also contributes descriptive data on body size and maturation parameters of Asian girls, who are rarely included in published studies.

A major advantage of DXA breast density, compared to mammography and other novel approaches, is that it is widely available in clinics and hospitals, and it is relatively inexpensive to obtain. Unlike mammography, DXA provides precise and accurate volumetric breast density and whole body soft tissue composition, both of which may be useful in assessing breast cancer risk. Magnetic resonance imaging (MRI), a method without radiation exposure, is also under investigation in young women. However, the higher cost and limited access to MRI, compared to DXA, will likely keep MRI from being a clinical standard (Boyd et al., 2009).

CONCLUSION

Breast density, as assessed by %FGV from DXA, was strongly negatively associated with body fat during adolescence among multiethnic adolescent girls; %FGV of the breast declined with higher % total body fat, as has been observed in adult women (Samimi et al., 2008). BMI was also highly correlated with DXA measures, which may have more clinical utility. Achieving

menarche positively improved prediction of %FGV to a similar, though stronger, degree than age.

This information will contribute to understanding needed to develop a routine method of breast density evaluation of young women and girls. Our findings suggest that DXA has the potential to provide a low-radiation option for evaluating breast density in adolescents and young women. This work is vital to support further development of a routine method of breast evaluation in young women to understand lifestyle factors and other determinants of breast development and to design future interventions to prevent breast cancer.

ACKNOWLEDGEMENT

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Table 1. Girls' characteristics by Tanner breast stage (T1-5)*

Characteristic	All (range)	T1 & 2	T3	T4	T5	r^	P value
N	113	17	38	13	45	---	---
Age (years)	13.9±1.7 (10.2-16.9)	12.0±1.6	13.5±1.6	14.3±1.4	14.8±1.3	0.52	< 0.0001
Weight (kg)	54.1±14.3 (32.2-105.8)	42.1±12.2	49.9±10.6	54.6±10.1	61.9±14.5	0.55	< 0.0001
Height (cm)	157.4±8.4 (136.7-178.5)	147.7±7.1	157.1±8.5	161.3±4.6	160.0±6.8	0.40	< 0.0001
BMI (kg/m^2)	21.7±4.9 (15.3-39.4)	19.1±4.0	20.2±3.4	21.0±3.8	24.2±5.6	0.46	< 0.0001
% Total body fat	30.1±9.1 (13.1-54.3)	27.3±9.7	27.4±7.4	30.6±8.1	33.2±9.6	0.27	< 0.01
TPFR	0.98±0.17 (0.65-1.44)	0.90±0.16	0.98±0.16	0.94±0.16	1.02±0.17	0.21	0.03
Breast volume (cm^3)	346.7±285.3 (26.1-1751.7)	109.9±80.9	229.1±123.0	324.0±132.0	541.9±338.4	0.70	< 0.0001
FGV (cm^3)	196.4±120.0 (18.5-579.1)	51.6±29.4	144.1±48.3	214.5±62.2	289.9±117.2	0.79	< 0.0001
%FGV	64.7±19.4 (21.6-99.2)	56.0±21.1	68.7±16.8	70.9±17.9	62.7±20.3	0.01	0.98

*Means and standard deviations except when specified
^Spearman rank order correlation coefficien

Table 2A. Stepwise regression model predicting %FGV with menarcheal status

Step	Effect entered	Estimate	SE	P value	Adjusted R^2
1	DXA body fat, %	-1.51	0.15	<0.0001	0.67
2	Achieved menarche	11.14	2.39	<0.0001	0.77
3	BMI z-score	-5.03	1.38	0.004	0.78
4	Tanner breast stage	2.35	0.99	0.02	0.79
5	Other ethnic group	3.44	1.84	0.06	0.80

Table 2B. Stepwise regression model predicting %FGV without menarcheal status

Step	Effect entered	Estimate	SE	P value	Adjusted R^2
1	DXA body fat, %	-1.64	0.17	<0.0001	0.67
2	Age, y	1.74	0.69	<0.0001	0.75
3	Tanner breast stage	3.54	1.06	0.004	0.76
4	BMI z-score	-3.10	1.63	0.13	0.76
5	Other ethnic group	3.42	1.96	0.08	0.77

Figure 1. Dual Energy X-ray Absorptiometry high resolution breast image of an Asian girl (Tanner breast stage 3, age = 12.6 years, 34th percentile of BMI-for-age)
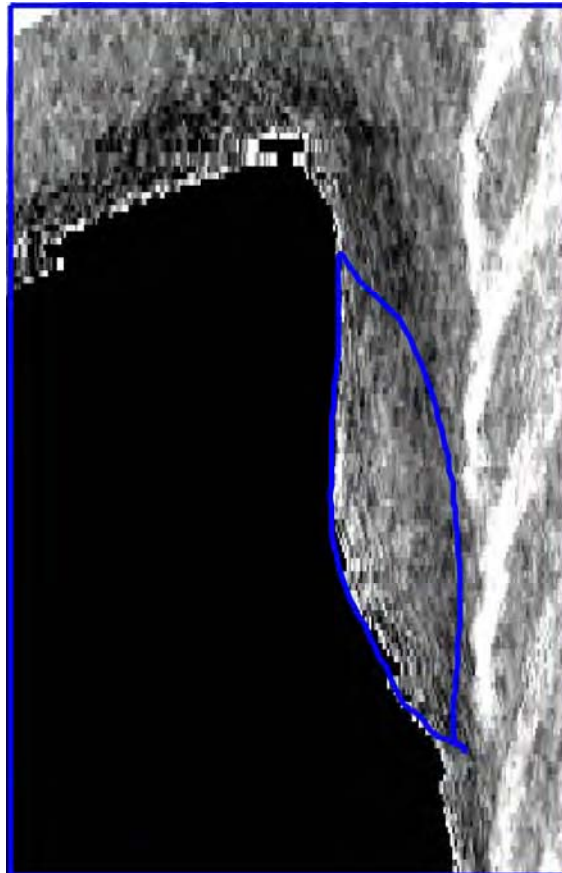
Figure 2-A. Breast volume (top series) and absolute FGV (bottom series)
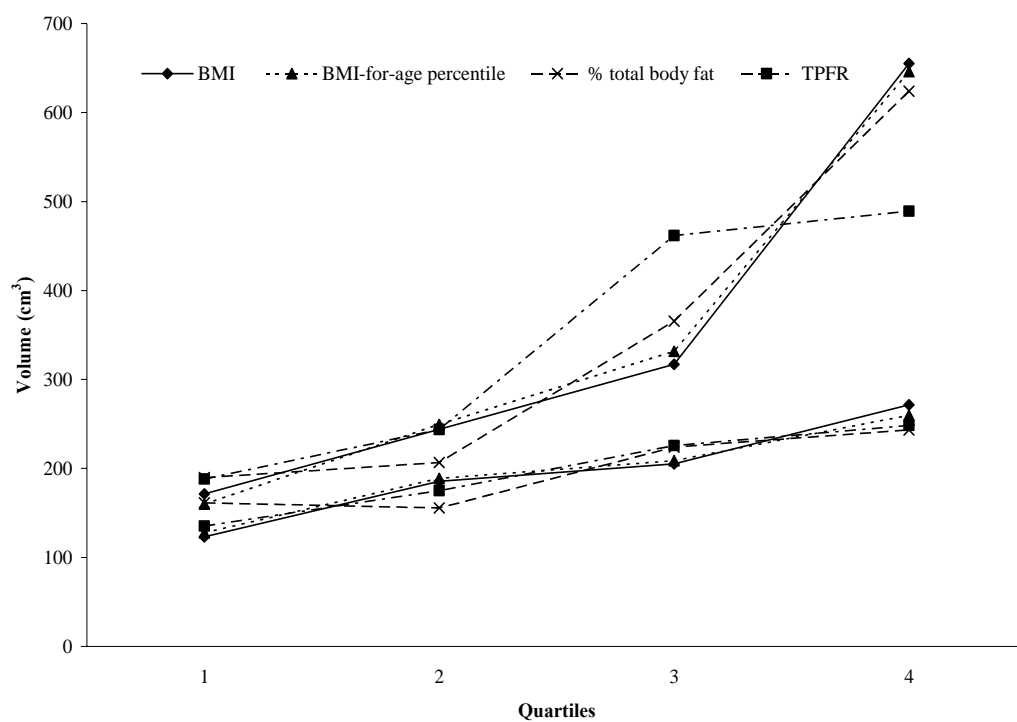by quartile of body fat variables

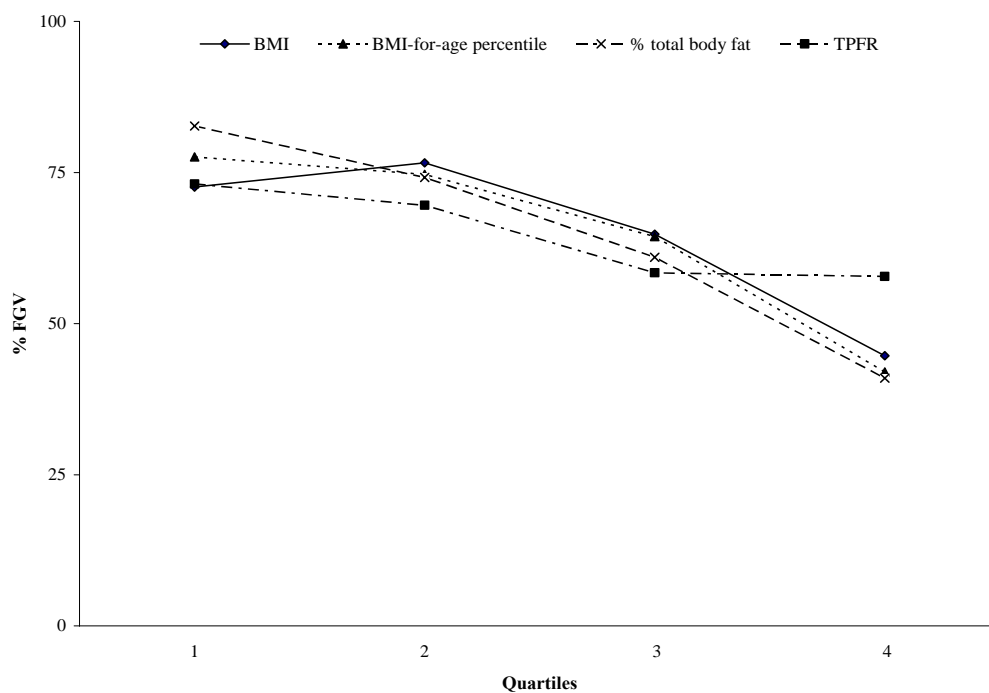Figure 2-B. Percent fibroglandular volume by quartile of body fat variables

Figure 3A. Breast density (%FGV) by tertile of % total body fat and ethnicity
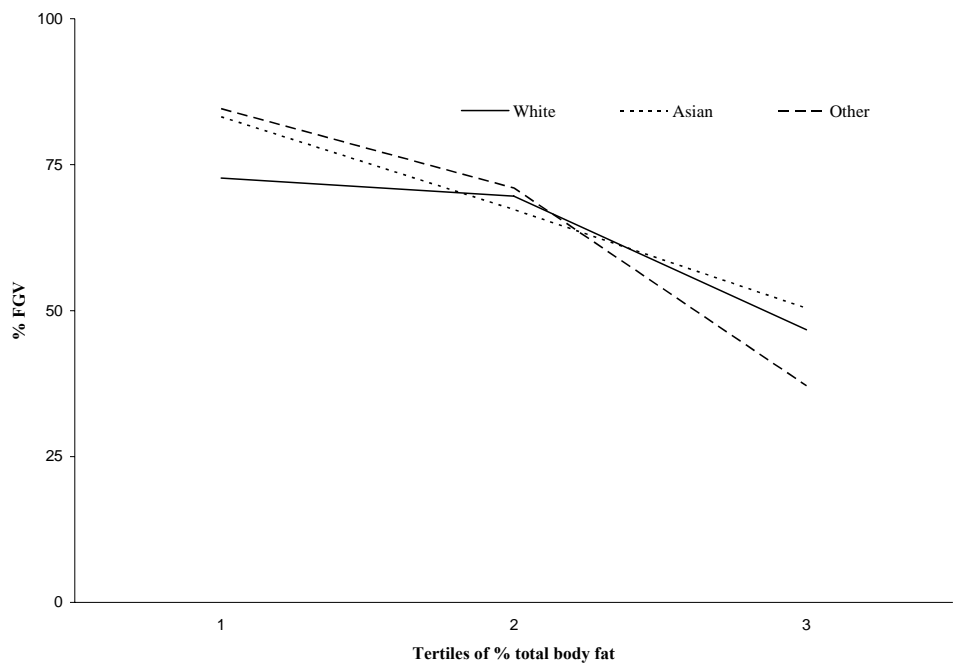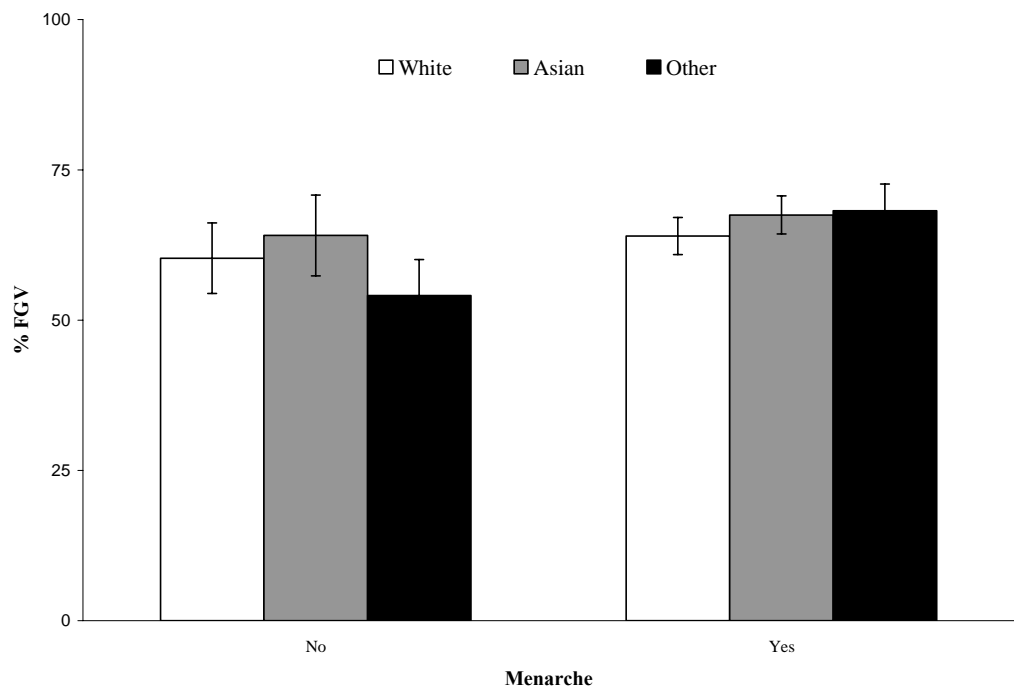

Figure 3B. Breast density (%FGV) by menarche and ethnicity

A comparison of breast density measures between mothers and adolescent daughters

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ABSTRACT

Introduction Based on the importance of breast density as a predictor of breast cancer risk, we examined the heritable component of breast measures in mothers and daughters using Dual Energy X-ray Absorptiometry (DXA).

Methods We recruited 101 mothers ≥30 years and their daughters aged 10-16 through Kaiser Permanente Hawaii. Scans of both breasts were taken using a clinical DXA system calibrated to distinguish fibroglandular and fatty breast tissue. We calculated Spearman correlation coefficients between mothers and daughters for breast area, volume, absolute fibroglandular volume (FGV), and %FGV and performed multiple linear regression to include relevant covariates.

Results We observed significant correlations of breast measures in mothers and daughters, especially for more mature girls with r = 0.41 for breast area (p <0.01), r = 0.33 for breast volume (p = 0.02), r = 0.49 for absolute FGV (p <0.0001), but no correlation for %FGV (r = -0.06, p = 0.65). These associations remained significant after adjustment for age, body mass index, ethnicity, and breast stage. Body mass index (BMI) and Tanner breast stages of the daughters were significantly associated with all breast measures except for %FGV that was inversely related to BMI.

Conclusions These results suggest that the heritability of breast volume and amount of dense tissue is already visible in adolescence, but an association for percent density may only become apparent later in life. Monitoring changes in breast tissue composition during adolescence and young women may add important information to understanding breast cancer risk later in life.

INTRODUCTION

Mammographic density, the distribution of fat, connective, and epithelial tissue in the female breast, has been used as a biomarker for breast cancer risk because a high percentage of dense parenchyma on mammographic images has been shown to confer a 4-6 fold higher risk for breast cancer [1]. However, it has been argued repeatedly that the absolute amount of dense tissue is the true determinant of breast cancer risk [2;3]. Ethnic differences in breast density have been described repeatedly; in particular, women of Asian ancestry were shown to have higher breast density than Caucasians due to their smaller breast size [4;5]. In addition to hormonal and anthropometric determinants, the proportion of the breast occupied by density, at a given age, is highly heritable [6]. Breast density was significantly correlated between sisters (r = 0.16-0.27) but not mother-daughter pairs (r = 0.02-0.11) in a cohort of affected families [7]. In a twin study, the correlation for the percentage of dense tissue was 0.63 for monozygotic pairs, but only 0.27 for dizygotic pairs suggesting that heritability accounted for 63% of variation in density [8]. Several studies also detected associations of breast density with variant alleles in genes involved in the regulation of mammographic density [9].

Given the relatively high radiation dose, screening mammograms are only recommended for women 40 years and older and cannot be used to monitor breast composition in young women and girls, as the risk outweighs potential benefits in that age group [10]. Therefore, Dual X-ray absorptiometry (DXA) has been explored as a research tool with the advantage that it is commonly available, is the reference standard for measuring whole-body composition, does not entail breast compression, and uses an X-ray dose 10 times lower than mammography [11;12]. DXA models breasts as containing two volumes of tissue, fibroglandular volume (FGV) and fat. This differs from mammographic density that is defined as the ratio of radiodense area to total area in the mammogram. We demonstrated in a pilot study of pubertal girls that all stages of breast development, as described by Tanner, can be imaged using DXA breast scans [13]. In a cross-sectional investigation, the correlation between mammographic and DXA density among adult women was 0.76 (p <0.0001). The present analysis is based on the hypothesis that, due to its strong genetic component, breast density from DXA scans is correlated between mothers and adolescent daughters. Thus, we analyzed the relationship between breast density measured by DXA in 101 mothers and 113 daughters who represented the major ethnic groups in Hawaii.

MATERIALS AND METHODS

Study design and procedure. The current analysis was conducted as part of a mother-daughter study that measured breast density and body-fat composition in adult women and their adolescent girls using DXA [14]. The project was approved by the Committee on Human Studies at the University of Hawaii and the Institutional Review Board of Kaiser Permanente (KP) Hawaii. We recruited women aged 30 years and older, who had received a normal mammogram (BIRADS categories 1 through 3) in the last 2 years, and their daughters aged 10-16 years through KP Hawaii, a large health maintenance organization. We mailed 3,915 invitation letters over 11 months (Figure 1). Potential participants were selected from the KP Hawaii membership data base according to the age and mammographic criteria. From the 304 respondents, we excluded mothers who had no mammogram, a previous history of breast cancer or surgery, an abnormal mammogram, a previous biopsy, breast implants, or chronic health condition that interfered with study participation. We excluded girls without breast development as well as mother-daughter pairs who were not biologically related or did not reside on the island of Oahu.

Of the 138 eligible mother-daughter pairs, 101 pairs plus 12 additional daughters completed the study visit. Prior to DXA scans, all participants signed informed consent, answered a demographic questionnaire, and completed height and weight measurements in duplicate. Body mass index (BMI) was calculated from measured height and weight and classified as normal (18.5 - <25), overweight (25 - <30) and obese ($\geq$30 kg/m^2) for mothers. In the demographic questionnaire, mothers and daughters reported the percentages of all ethnic backgrounds that applied to their parents. The questionnaire also inquired about reproductive factors, such as age at menarche and number of children.

DXA data collection. At the exam, a urine test excluded pregnancy in all participants. We performed DXA scans of both breasts, as well as of the whole body, using the research scan protocol and software version 10.1 on a GE Lunar Prodigy Bone Densitometer (GE Healthcare). Prodigy utilizes an ultra low radiation and cadmium-zinc-telluride detector to convert X-rays into an electronic signal without the intermediate conversion to light. We used a custom thickness-step phantom made of reference materials to recalibrate the DXA device as described in detail elsewhere [11;15]. After changing into a hospital gown, both breasts were scanned in the decubitus mediolateral position with the nipple positioned in a true lateral profile. A duplicate breast scan of the left breast was performed on a random 10% of subjects for quality control.

A low-energy and high-energy attenuation image was saved for each scan using the options available from GE Lunar for the research scan mode. These images were analyzed at the University of California at San Francisco, using a Breast Density Workstation. The total projected breast area was manually delineated on each image by the same operator. From the bottom of the breast, the delineation followed the thoracic cage, then the pectoral muscle to continuously reach the exterior of the breast. Finally the external line was delineated. To calculate the DXA density, a two-compartment model of adipose and fibroglandular tissue was used [11]. Scans of a calibrated phantom with known composition and thicknesses allowed the calculation of calibration curves. Percent fibroglandular volume density (%FGV), FGV, total breast area, and breast volume were computed.

For quality control, a calibrated phantom was scanned over 8 months, once every day that participants were scheduled and once a week if no participant was scheduled. The phantom varied in thickness (2, 10, and 20 cm) and contained three %FGV values of 28%, 65%, and 100%. The phantom precision values ranged from 1.9% (10 cm in thickness and 65% composition) to 5.4% (2 cm in thickness and 65% composition). For the repeated breast scans, a root mean square standard deviation of 2.5 and a correlation of 0.975 were achieved.

Statistical Analysis. All statistical analyses were performed using the SAS statistical software package version 9.2 (SAS Institute, Inc., Cary, NC). To evaluate the association of breast measures between mothers and daughters, we calculated Spearman correlation coefficients for breast area, volume, absolute FGV, and %FGV. In addition, we performed multiple linear regression analyses to include covariates. The breast measures of the girls were modeled as the dependent variable, while Tanner breast stages, ethnicity, age, BMI, as well as the characteristics of the mothers were treated as independent variables. Because of the small sample size, ethnicity was classified as three binary categories: all or part Caucasian, all or part Asian (Japanese, Chinese, Filipino, Korean, Other Asian), and all or part Other (Hawaiian and other Pacific Islanders, Black, Native American, Hispanic, Other). If a participant reported Caucasian and Other, she was assigned "1" for Caucasian and Other and "0" for Asian. Because 12 mothers had

2 participating daughters, we repeated the models with only one of the two daughters at a time to remove the correlation associated with duplicate maternal information.

RESULTS

The mean age of the mothers was 47.7±4.8 years and that of the daughters was 13.9±1.7 years (Table 1). The mean BMI of the mothers was 27.5±6.0 kg/m^2; 59% of the mothers were overweight (N = 33) or obese (N = 27). The mean BMI for the daughters was 21.7±4.9 kg/m^2 and was correlated with the BMI of the mothers (r = 0.25; p <0.01), though only 27% were overweight (N = 15) or obese (N = 15) by BMI-for-age percentile criteria [16]. At the time of the study, 17 girls were in Tanner breast stages 1&2, 38 in stage 3, and 58 in stages 4&5; 80 girls had reached menarche. As is typical in Hawaii, 38% of the mothers reported more than one ethnicity and 64% of the girls reported at least two ethnic backgrounds.

As expected, mean total area, total volume, and absolute FGV were higher in mothers than in daughters, while mean %FGV was higher in daughters than in mothers (Table 2). As illustrated in Figure 2, the median and the variance, as measured by the interquartile range (IQR), were greater in daughters than in mothers. We observed statistically significant correlations between breast measures of mothers and daughters with 0.26 for breast area, 0.22 for breast volume, 0.32 for absolute FGV, but no correlation for %FGV (r = -0.05). Except for %FGV, the association was stronger for girls who had reached Tanner breast stages 4&5 (Figure 3); the correlations were 0.41 for breast area, 0.33 for breast volume, 0.49 for absolute FGV. BMI was strongly correlated with DXA breast measures: 0.70 for breast area, 0.76 for breast volume, 0.57 for absolute FGV and -0.58 for %FGV (p <0.0001 for all), as were the correlations of Tanner stage with breast area (r = 0.73, p <0.0001), breast volume (r = 0.69, p <0.0001) and absolute FGV (r = 0.79; p <0.0001). However, %FGV was not significantly associated with Tanner stage (r = 0.04; p = 0.71). Analyses that included only one daughter produced similar results.

In multiple regression models of all mother-daughter pairs (Table 3), breast volume (p = 0.03) and absolute FGV (p <0.01) of the mothers remained modestly associated with the respective measures of their daughters, but no association was observed for %FGV (p = 0.82). In the adjusted models, BMI and Tanner breast category of the girls were significantly associated with breast volume and absolute FGV (Table 3). Whereas %FGV increased with higher Tanner breast stages, BMI was inversely associated with %FGV. Reporting any Other ethnicity was related to higher %FGV (p = 0.04); otherwise ethnicity was not significant. Additional analyses by ethnic group showed little difference in the effect for %FGV; the estimate was 0.004 (p = 0.98) in Caucasians; 0.02 (p = 0.87) in Asians; and 0.17 (p = 0.37) in Others. When limited to the more mature girls with Tanner breast stages 4&5, the regression estimates for breast volume and absolute FGV of the mothers improved to 0.18 and 0.63, while the estimate for %FGV was -0.24 (p = 0.20). After stratification by BMI status, this non-significant inverse association was limited to overweight and obese mothers (ß = -0.16; p = 0.45) and was not observed in normal-weight mothers (ß = 0.03; p = 0.88).

DISCUSSION

In this comparison of DXA-based breast measures among biological mother-daughter pairs, modest correlations were observed for breast size and for the amount of fibroglandular tissue in the breast, but not for %FGV, which is the ratio of fibroglandular tissue to total volume, an equivalent to percent mammographic density. The associations were stronger for girls in Tanner breast stages 4&5, probably because they were closer to reaching their final breast size.

As with mammographic density, BMI was inversely associated with DXA-based percent density in mothers and daughters, but Tanner stage was the major predictor for girls. These results suggest that the heritability of breast volume and amount of dense tissue is already visible in adolescence, but an association for %FGV may only become apparent at a later time.

The limited literature on the heritability of breast density did not include any girls as young as those in this report. A familial study observed a modest correlation (r = 0.27) among sisters and a non-significant association between mothers and adult daughters [7]. Our results conflict with the moderate correlation (r = 0.28) observed in an investigation of mothers and daughters that used magnetic resonance imaging (MRI) [17], though the discrepancy may be due to age differences. Since the daughters in the MRI study were older (20.8±4.9 years) than the girls in the current study (13.9±1.7 years), their breast maturation had ended, whereas the younger girls are subject to great variability in the dense to non-dense composition of the breast until breast development reaches completion in early adulthood. In addition, the MRI study used an unorthodox measure of breast density, percent water volume, and in doing so combined all cellular volume into the non-dense compartment.

On the other hand, our findings agree with the distribution patterns of density in the MRI investigation, which described a larger variance in percent density among daughters than mothers, in particular among younger daughters (15-18 years) [17]. Our data from even younger girls support the hypothesis proposed by Boyd et al. in 2009 that the median density and the variance of density decrease with age; the median %FGV and IQR were both higher for our young girls than for the older adolescents in the MRI study. Similar to the current results, our pilot study of 13-14 year old girls also described increases in breast volume and absolute FGV by Tanner breast stage but did not observe a clear trend for %FGV [13]. Evidence from histology studies indicates that pubertal breast development involves changes in both the epithelium and the stroma [18]. The amount of fibrous and fatty tissue increases, which is dense and fatty, i.e., non-dense, breast tissue. The extension of ducts is preceded by proliferation of connective tissue, but the two cannot be distinguished by imaging methods. Given the problems of examining adolescent breast tissue, the timing of the full development of the breast gland is not well understood, but full maturation of the breast takes several years after menarche [19]. Thus, understanding the magnitude of genetic influence from mothers is limited in this age group and requires more information on the breast-tissue composition during the transitional phase of adolescence through early adulthood.

Strengths of the present study include the application of a new DXA technique that was previously tested in adolescent girls of all Tanner breast stages [13]. A major limitation of the current study is the relatively small sample size that did not allow separate analyses by ethnicity. Although not statistically significant, the higher percent density among Asians agreed with previous studies among adult women [4]. Error in measuring the smaller size breasts in Tanner stages 1&2 may be responsible for underestimates of correlations. As in the familial study, genetic influences on breast density may be more difficult to detect in mothers and daughters than between sisters due to confounding by age and adiposity as well as hormonal and environmental influences [7].

In this investigation, both the size of the developing breast and the amount of dense breast tissue were correlated between pubertal girls and their mothers. The reasons for a lack of correlation in percent density are not clear, but may due to the young age of the girls whose growth will continue several more years or due to confounding by maternal obesity. Since the amount of absolute breast density shows a similar association with breast density as percent

mammographic density [2;3], the correlation in absolute fibroglandular tissue supports a heritable component of breast density that may be useful in risk prediction models [20;21]. Future investigations using a longitudinal design and examination of siblings may be able to elucidate the changes in breast composition in this age group. The ability to examine breast tissue development during adolescence allows us to investigate risk factors, e.g., nutrition, adiposity, physical activity patterns, that influence breast cancer risk later in life [22-24].

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
GM and RN conceived the research idea, obtained funding, designed the study, and oversaw the data collection. JS developed the DXA imaging method and directed the analysis of the breast scans. YM and YD coordinated the recruitment and data collection. YM performed the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Table 1. Characteristics of mothers and daughters

		Mothers	Daughters
N		101	113
Age (years)		47.7±4.8	13.9±1.7
Weight (kg)		70.8±17.0	54.1±14.3
Height (cm)		160.3±7.3	157.4±8.4
BMI (kg/m^2)		27.5±6.0	21.7±4.9
Age at menarche (years)		12.5±1.6	11.7±1.1[†]
Number of children		2.5±1.0	---
Ethnicity, all or part (N)*	Caucasian	49	77
	Asian	64	83
	Other	33	53
Tanner stage for breast development (N)	1-2		17
	3		38
	4-5		58

*Based on percentages; participants were counted more than once if they reported multiple ethnicities.
[†]N=80 who had reached menarche

Table 2. Breast measures for mothers and daughters

DXA measures	Mothers	Daughters	r*	p value
All mother-daughter pairs				
N	101[†]	113		
Total area (cm^2)	92.0±38.9	43.2±26.4	0.26	<0.01
Total volume (cm^3)	820.3±494.1	346.7±285.3	0.22	0.02
FGV (cm^3)	272.5±107.6	196.4±119.9	0.32	<0.001
%FGV	38.9±14.1	64.7±19.4	-0.05	0.63
Mothers and daughters in Tanner stages 4&5 only				
N	54[†]	58		
Total area (cm^2)	101.2±41.4	58.7±26.7	0.41	<0.01
Total volume (cm^3)	908.5±529.3	493.1±.317.0	0.33	0.02
FGV (cm^3)	297.7±114.8	273.0±111.5	0.49	<0.0001
%FGV	37.9±12.8	64.6±20.0	-0.06	0.65

*Spearman's Correlation Coefficient
[†]Mothers with two daughters were included twice.

Table 3. Regression models to predict the daughters' breast measures based on their mothers' measures and covariates

			Breast volume		FGV		%FGV	
			Estimate	p value	Estimate	p value	Estimate	p value
All mother-daughter pairs (N = 113)[*]								
Corresponding Mother's DXA measure			0.11	0.03	0.30	<0.01	-0.03	0.82
Ethnicity (all or part), daughter[2]	Caucasian	N = 77	9.56	0.78	-8.92	0.60	-2.79	0.32
	Asian	N = 83	-13.54	0.70	8.25	0.65	3.45	0.24
	Other	N = 53	4.28	0.88	22.38	0.13	5.13	0.04
BMI, daughter (kg/m^2)			41.16	<0.0001	6.30	<0.001	-3.30	<0.0001
BMI, mother (kg/m^2)			-5.77	0.17	-2.43	0.13	0.14	0.62
Tanner breast (1&2, 3, 4&5)[‡]			82.29	<0.01	80.50	<0.0001	8.41	<0.001
Age, daughter (year)			10.53	0.31	8.34	0.11	1.64	0.06
Age, Mother (year)			4.63	0.15	2.44	0.14	-0.15	0.59
Mothers and daughters in Tanner stages 4&5 only (N = 58)								
Corresponding Mother's DXA measure			0.18	0.05	0.63	<0.0001	-0.24	0.20
Ethnicity (all or part), daughter[†]	Caucasian	N = 40	-50.18	0.40	-33.92	0.24	-2.21	0.55
	Asian	N = 40	-35.67	0.55	12.82	0.65	2.86	0.44
	Other	N = 28	7.20	0.88	34.30	0.17	4.37	0.18
BMI, daughter (kg/m^2)			47.33	<0.0001	5.66	0.02	-3.20	<0.0001
BMI, mother (kg/m^2)			-11.44	0.16	-6.24	0.03	0.18	0.64
Age, daughter (year)			22.19	0.30	3.33	0.75	0.25	0.85
Age, Mother (year)			3.90	0.53	5.63	0.07	-0.14	0.72

[*]Twelve mothers with two daughters were included twice.
[†]Based on percentages; participants were counted more than once if they reported multiple ethnicities.
[‡]Tanner breast stages were grouped into 1&2, 3 and 4&5 as a continuous variable.
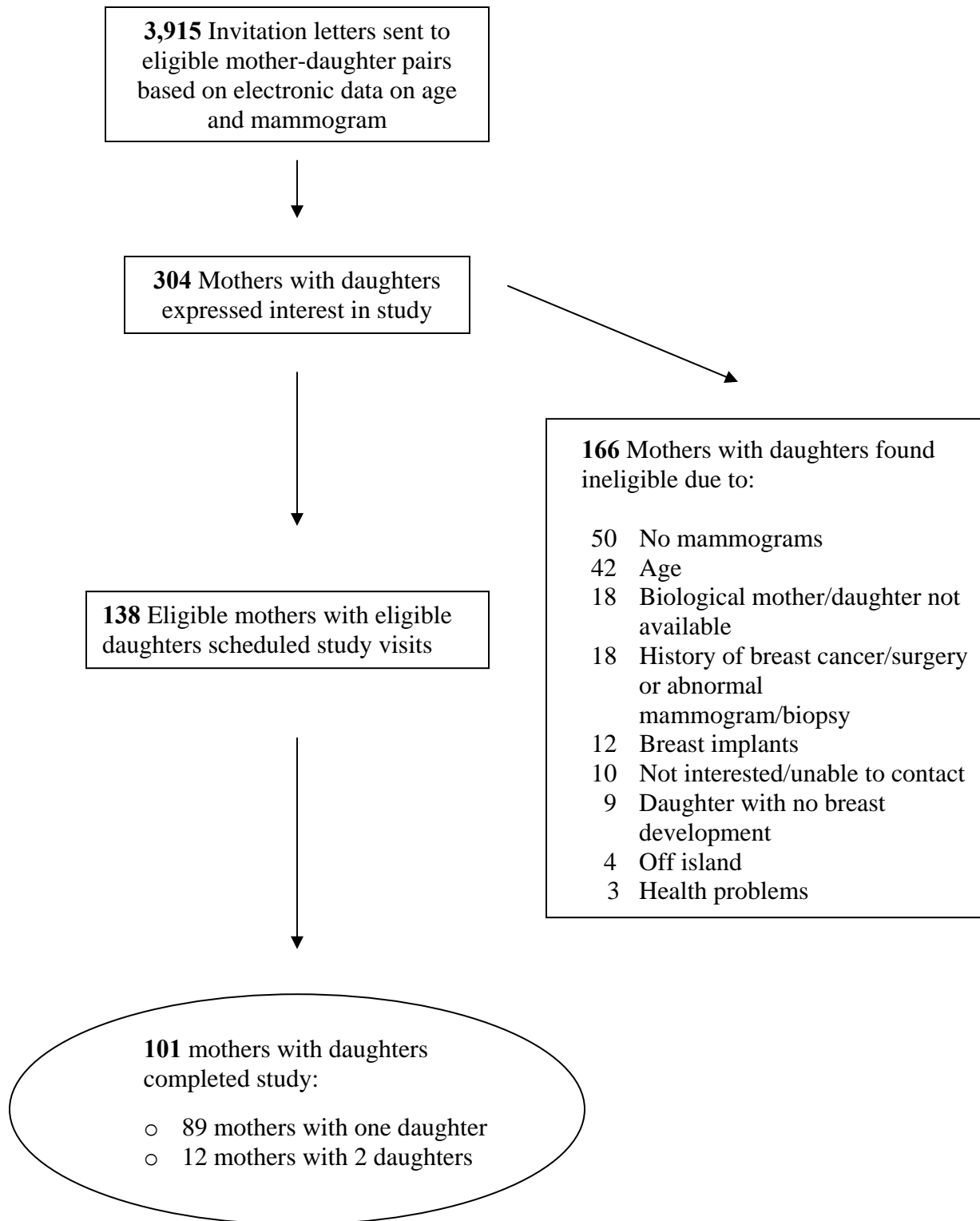
Figure 1. Recruitment and study population

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┌─────────────────────────────────┐
│ 3,915 Invitation letters sent to │
│ eligible mother-daughter pairs   │
│ based on electronic data on age  │
│ and mammogram                    │
└─────────────────────────────────┘
                │
                ▼
┌─────────────────────────────────┐
│ 304 Mothers with daughters       │─────────┐
│ expressed interest in study      │         │
└─────────────────────────────────┘         │
                │                            ▼
                │         ┌──────────────────────────────────────┐
                │         │ 166 Mothers with daughters found     │
                │         │ ineligible due to:                   │
                │         │                                      │
                │         │  50  No mammograms                   │
                │         │  42  Age                             │
                ▼         │  18  Biological mother/daughter not  │
┌─────────────────────────────────┐│      available               │
│ 138 Eligible mothers with eligible││  18  History of breast cancer/surgery│
│ daughters scheduled study visits ││      or abnormal             │
└─────────────────────────────────┘│      mammogram/biopsy        │
                │         │  12  Breast implants                 │
                │         │  10  Not interested/unable to contact│
                │         │   9  Daughter with no breast         │
                │         │      development                     │
                │         │   4  Off island                      │
                │         │   3  Health problems                 │
                ▼         └──────────────────────────────────────┘

  ╭───────────────────────────────────╮
  │ 101 mothers with daughters         │
  │ completed study:                   │
  │                                    │
  │  o  89 mothers with one daughter   │
  │  o  12 mothers with 2 daughters    │
  ╰───────────────────────────────────╯
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Figure 2. Distributions of DXA percent density (%FGV) in mothers (A) and daughters (B)

A. Mothers

Median = 35.8 (arrow)
Interquartile range = 18.5

Mothers' % FGV

B. Daughters

Median = 69.4 (arrow)
Interquartile range = 27.8
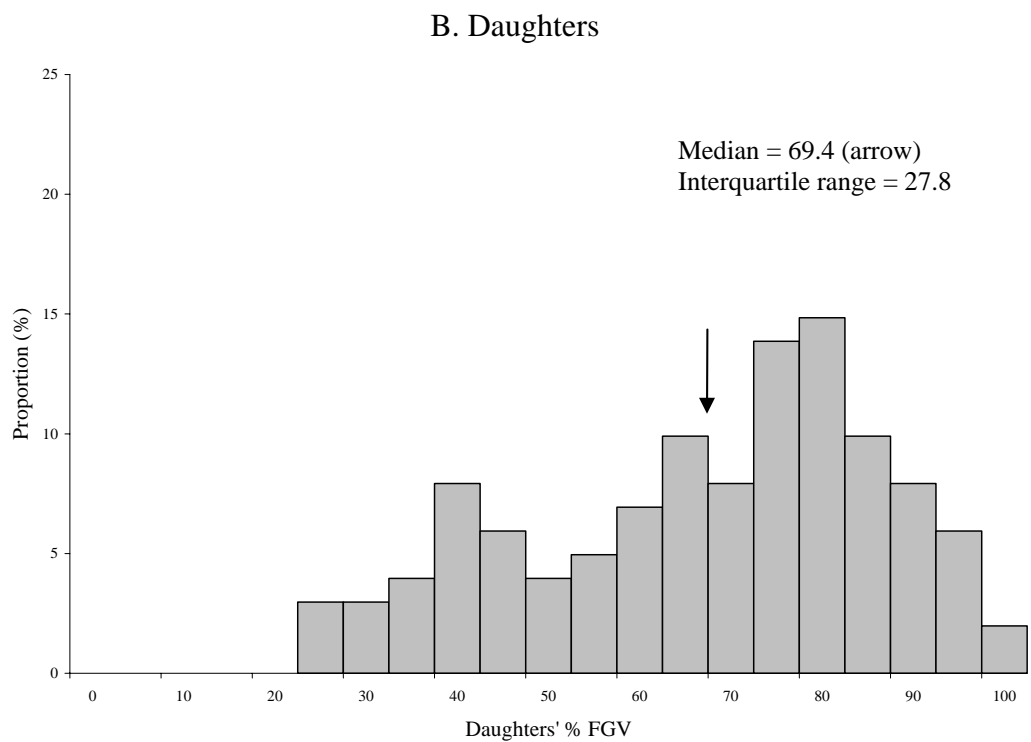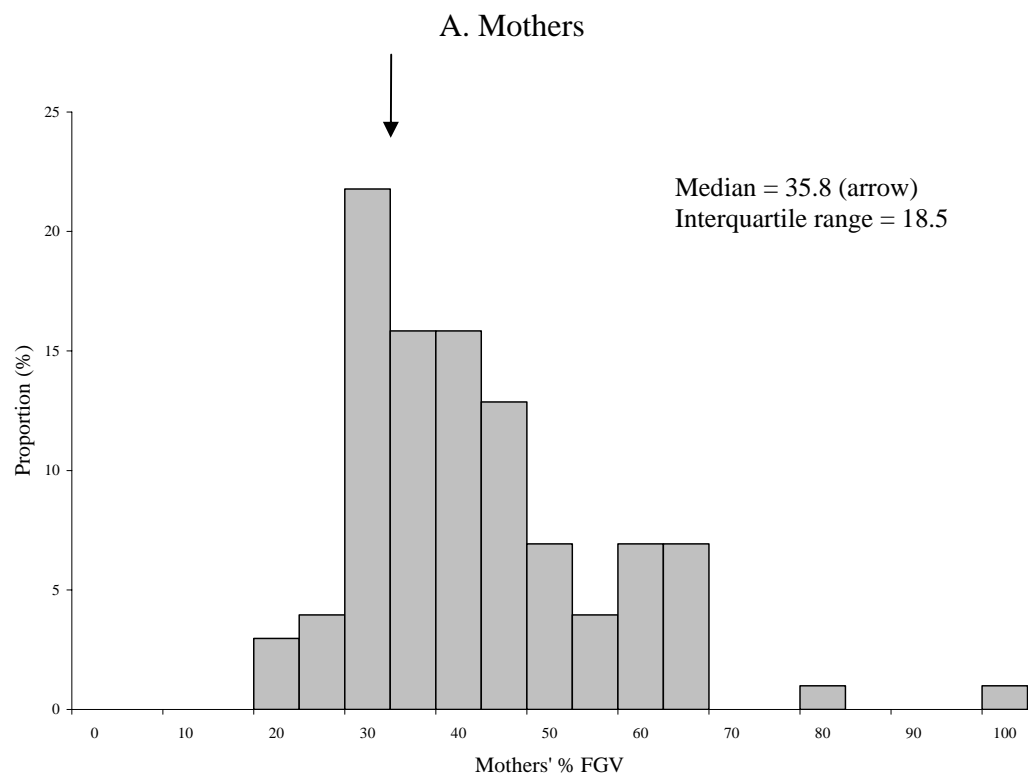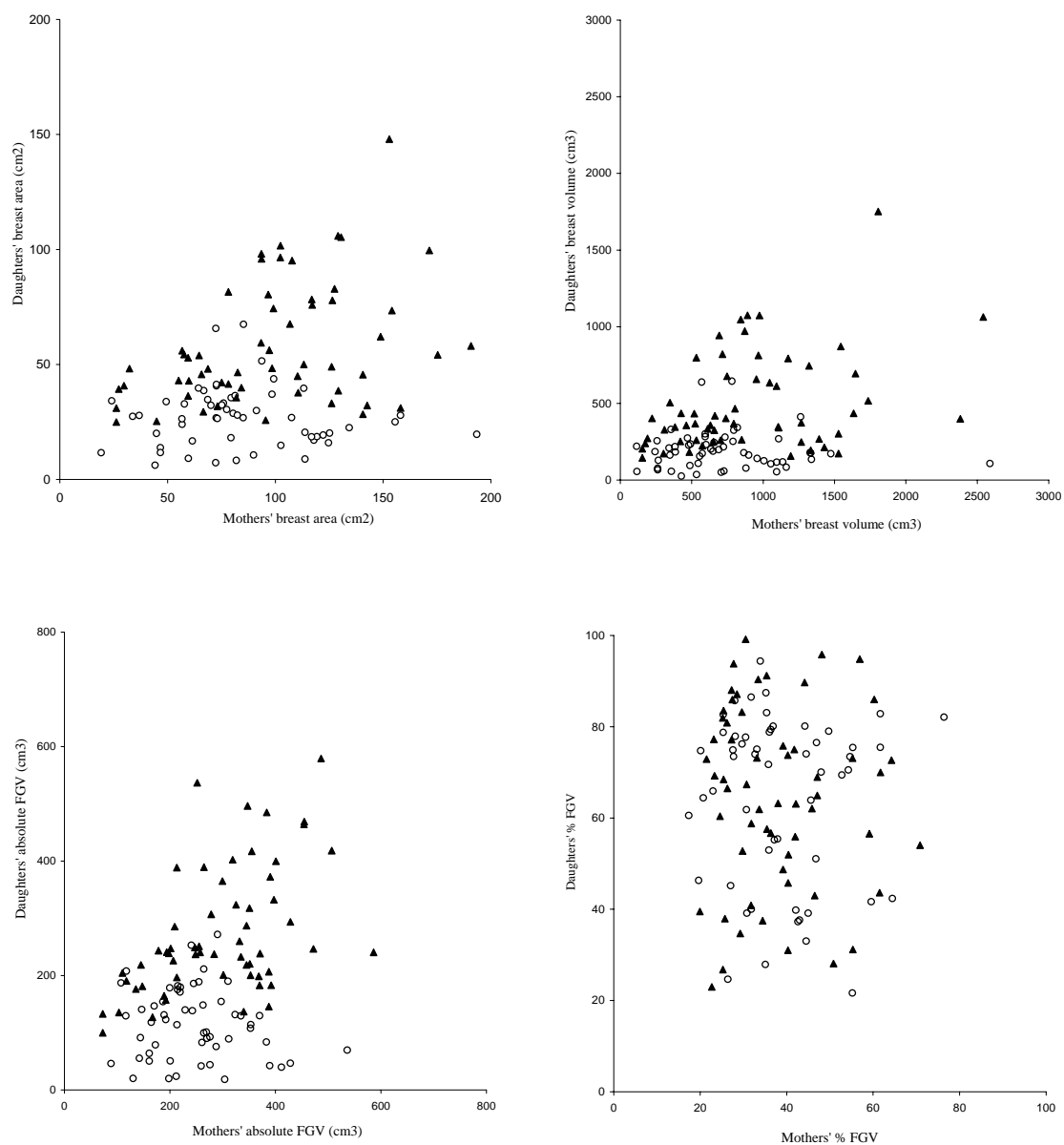
Daughters' % FGV

Figure 3. Association of breast measures between mothers and daughters

○: Tanner breast stages 1, 2 and 3   ▲: Tanner breast stages 4 and 5